



Effect of Flaxseed Supplementation in the Maternal Diet During Early Lactation on the Oxidative Status of Sows and Growth of Piglets

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ABSTRACT

The present study was conducted on 21 crossbred Large White Yorkshire sows to assess the effect of flaxseed supplementation on oxidative stress in sows and growth of piglets. All sows were equally allocated into three groups. Group 1 served as the control and followed their normal feeding schedule. Group 2 and group 3, in addition to their normal feeding schedule, were supplemented with flaxseed at a rate of 0.5% and 1.0% of the dry matter, respectively. Blood samples were collected 15 days prior to farrowing, on the day of farrowing (day 0), at weekly intervals until day 28 of lactation and at monthly intervals during gestation to assess the oxidative status in each sow. Body weight and weight gain in each piglet was measured during lactation period. The assessment of oxidative stress in blood was done through estimations of malondialdehyde (MDA), end product of lipid peroxidation. No changes ($P > 0.05$) in MDA concentrations were manifested at any of the days measured during lactation and gestation in sows of all the groups. Similarly, there was no effect of flaxseed treatment on superoxide dismutase (SOD) levels in all sows at any of the days measured during lactation period ($P > 0.05$) as well as during gestation period (days 30-90). In the current farrowing, the piglets exhibited a steady increase ($P < 0.05$) in bodyweight as well as weight gain in all groups from day 0 until day 28 of lactation. No effect ($P > 0.05$) of flaxseed fed to sows on litter weight and weight gain was noticed until day 21 of lactation. However, the effect of treatment was evinced on day 28 of lactation compared to control group. In conclusion, flaxseed supplementation had no impact on MDA and SOD concentrations in sows; nevertheless a treatment effect was noticed on body weight and weight gain in piglets.

Keywords: Flaxseed, Malondialdehyde, Oxidative stress, Sow, Superoxide dismutase.

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INTRODUCTION

Polyunsaturated fatty acids (PUFA) are essential components of lipids required for optimal growth and development of piglets. Supplementation of PUFA in sow lactation diets, can lead to increased secretion of PUFA in milk, essential to support growth and development of nursing litter (Odleet *et al.*, 2014). Therefore, including PUFA in the sow diet may have beneficial effects on productive traits. Alternatively, the growing piglet leads to increased metabolic burden on sow, causing elevated systemic oxidative stress during lactation (Kim *et al.*, 2013). Moreover, PUFA provide substrates for lipoperoxidation and further lead to oxidative stress as a result of their double bonds in structure (Shen *et al.*, 2015). Since cell membranes are rich in PUFA, oxidative damage can lead to altered transmission of signals within and between different cells and loss of membrane integrity (Su *et al.*, 2017). Hence, a good knowledge of fatty acid metabolism, transfer and possible negative physiological effects is need of the hour. Currently, there are no clear recommendations regarding the source and amount of PUFA supplementation. PUFA can be included in diet in the form of fatty acid precursor that can be converted to long chain derivatives by the animal itself. A possible source of fatty acid precursor is flaxseed, which is rich in α -linolenic acid. Flaxseed contains high (58% of the total fatty acids) content of α -linolenic acid, provides better palatability compared to fish meal owing to non-fishy odor, thereby making it an energy dense replacement compared to other feed ingredients (Erasmus, 1993). Flaxseed is used as a source of long-chain n-3 PUFA by providing Eicosapentaenoic acid (EPA; 20 5n-3) and Docosahexaenoic acid (DHA; 20 6n-3) and has been reported to restore antioxidant capacity because of the high content of EPA and DHA (Richard *et al.*, 2008). Until now, most studies (Bazinet *et al.*, 2003; De Quelen *et al.*, 2010) that investigated the effect of PUFA in maternal diet did not consider its implications on oxidative stress. Hence, the goal of this study was to focus on some physiological aspects that have been somewhat overlooked in the past. Therefore, the current study was undertaken to investigate the effect of dietary flaxseed to the maternal diet on antioxidant status in sows.

MATERIALS AND METHODS

Animals, experimental design and dietary treatments:

The study was approved by the Institutional Animal Ethics Committee (IAEC; GADVASU/2019/IAEC/50/01) of Guru Angad Dev Veterinary and Animal Sciences University. Twenty one apparently healthy pleuriparous

(2nd to 5th parity) pregnant (>95 days) crossbred Large White Yorkshire maternal-line sows were randomly maintained at pig farm, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana (n=6) and at private organized pig farm (Polar Pig Breeding Farm, Khassi Kalan, Ludhiana, n=15) located in peri-urban areas of Ludhiana. All sows at both farms were maintained under uniform feeding schedule (fed twice a day) with free access to drinking water and were kept under semi-loose housing system with average daily temperature of $23.3 \pm 1.1^\circ\text{C}$ and relative humidity of $71.2 \pm 1.0\%$ inside the pig sheds.

In the present study, all sows were considered starting at day 1 of current farrowing until the day of subsequent farrowing (days 150-155). The sows were allocated equally to three treatments viz. group 1, group 2 and group 3 based on parity, body weight at farrowing and number of piglets born. Accordingly, group 1 sows (n=7) served as control and followed their normal feeding regime starting on day 1 of lactation until the day of subsequent farrowing (days 150-155). Sows of group 2 (n=7) and group 3 (n=7), in addition to their normal feeding schedule, were supplemented flaxseed at a rate of 0.5 percent and 1.0 percent of the dry matter (Tanghe *et al.*, 2015), respectively, throughout the study period.

In farrowing pen (one sow/pen/group), each sow was offered standard lactation ration using maize, soyabean meal, de-oiled rice bran, wheat bran, salt and mineral mixture at the rate of 4%/kg bodyweight/day/sow, starting on day 1 of lactation, throughout the 28-day lactation period. Cross-fostering of piglets was done within a group, if required, immediately after 24 h post-farrowing to allow adequate colostrum intake from sows and to ensure their uniform nourishment. Litter size was standardized to 10 piglets per litter (10.0 ± 0.2 piglets). During the experimental period, piglets did not have access to creep feed and/or supplemental milk. Litters were weaned on day 28 of lactation. After weaning, the sows were shifted to a common breeding area and housed as breed groups (2-3 sows/pen/group) and fed above-mentioned lactation diets at the rate of 3%/kg bodyweight/day/sow (Table 1). In addition, each sow of group 2 and group 3 was continued flaxseed supplementation.

Abbreviation: BDL-Below Detection Limit; FS-Flaxseed supplementation

During the first three days after weaning, each sow was provided exposure to a rotation of mature boars daily for 30 minutes to facilitate estrus detection and checked for estrus signs, twice daily from day 4 until day 7. About six to eight hours following exhibition of physical signs of first standing estrus, the sows were bred with proven fertile boars. The sows were checked for pregnancy at day 25 after

Table 1. Nutritional composition of various diets fed to sows.

Item	Control diet (Group 1)	FS, 0.5% (Group 2)	FS, 1.0% (Group 3)
Ingredient, kg/100 kg			
Maize	48.0	48.0	47.5
Soybean meal	11.1	11.1	11.1
Rice polish	10.6	10.1	10.1
Deoiled rice bran	10.5	10.5	10.5
Groundnut extraction	18.5	18.5	18.5
Common salt	0.3	0.3	0.3
Specific mineral mixture	1.0	1.0	1.0
Crushed flaxseed	0.0	0.5	1.0
Chemical composition (analyzed)			
Crude fiber (%)	5.14	5.19	5.22
Crude protein (%)	20.61	20.65	20.73
Moisture (%)	9.37	9.55	9.18
ME (Kcal/kg)	3285	3290	3294
Total ash (%)	6.06	6.08	6.11
Ether extract (%)	5.11	5.11	5.23
Phosphorus (%)	0.74	0.81	0.83
Potassium (K, %)	0.36	0.41	0.34
Calcium (%)	1.22	1.27	1.35
Salt (%)	0.62	0.62	0.62
Vitamin A (IU/kg)	2497	2689	2644
Vitamin E (IU/kg)	38.17	38.42	38.38
Methionine (mg/100g)	0.47	0.53	0.58
Lysine (mg/100g)	0.83	0.90	0.96
Aflatoxin B1 (µg/kg)	BDL	BDL	BDL
Aflatoxin B2 (µg/kg)	BDL	BDL	BDL
Aflatoxin G1 (µg/kg)	BDL	BDL	BDL
Aflatoxin G2 (µg/kg)	BDL	BDL	BDL
C18:1 Oleic acid (per 100g fat)	1.09	1.16	1.20
C18:2 Linoleic acid (per 100g fat)	0.18	0.40	0.43
C18:3 Linolenic acid (per 100g fat)	0.07	0.39	0.45
C20:4 Arachidonic acid (per 100g fat)	0.86	0.91	1.05
C20:5 Cis-5,8,11,14,17-Eicosapentanoic acid (per 100g fat)	0.04	0.07	0.07
C22:6 Cis-4,7,10,13,16,19-Dicosahexanoic acid (per 100g fat)	0.02	0.05	0.05

breeding using ultrasound machine. Sows confirmed pregnant were housed in gestation pens (2-3 sows/pen/group). During gestation, sows were offered standard gestation diet at the rate of 3%/kg bodyweight/day/sow (Table 1). The amount of feed was rescheduled fortnightly according to the weight of sows. One week prior to expected date of farrowing, the pregnant sows were shifted to individual farrowing pens.

Collection of blood samples: Following short-time nose-snare restraint, blood samples (5 mL) were collected into heparinized (1:1000) polystyrene tubes from each sow through peripheral ear vein. Blood samples were collected fifteen days prior to farrowing (day 15), at farrowing (day 0), at days 7, 14, 21, 28 of lactation period and at days 30, 60 and 90 of gestation period to assess antioxidant status of sows. Blood samples were centrifuged for 15 minutes at

3000 rpm to harvest the plasma. The plasma samples were stored at -20°C until assayed.

Oxidative stress estimation in blood plasma:

Changes in antioxidant status were measured by analysis of lipid peroxidation (LPO) and superoxide dismutase (SOD) from blood samples collected fifteen days prior to farrowing (day -15), at farrowing (day 0), at days 7, 14, 21, 28 of lactation period and at days 30, 60 and 90 of gestation period.

Anti-oxidant status in blood: The assessment of oxidative stress in blood was done through estimations of malondialdehyde (MDA), end product of LPO and SOD activity.

Preparation of RBC Lysate for LPO: Exactly 1.0 ml blood was transferred to a 15 ml conical centrifuge tube and filled to capacity with fresh cold RBC lysis buffer. The mixture was shaken for 10 minutes at room temperature on a shaker to obtain clear red liquid. The liquid was centrifuged at 4°C for 10 minutes at 5000 rpm. The supernatant was decanted and pellet was suspended in 2.0 ml cold phosphate buffer saline (pH 7.2) and stored at -20°C, pending analysis.

Estimation of hemoglobin (Hb): In the central tube of hemocytometer, 100µl of 0.1N HCL was poured followed by addition of 20µl blood sample. The distilled water was added drop wise till the color of the tube matched with the side walls of the hemocytometer and Hb content in g% was recorded.

Assessment of MDA: Briefly 200 µl RBC lysate was incubated with 0.2 ml 150 Mm Tris HCl for 20 minutes at 37°C. After incubation, 1.0 ml TCA (10%) and 2.0 ml TBA (0.375%) were added and kept in boiling water bath for 20 minutes. Thereafter, mixture was cooled and centrifuged for 15 minutes at 5000 rpm. The supernatant was taken out and absorbance was read at 532 nm. The molar extinction coefficient for MDA is $1.56 \times 10^5 \text{ M}^{-1} \cdot \text{cm}^{-1}$.

CALCULATIONS

MDA (µmol/g Hb) =	OD × Volume of assay mixture × Extinction coefficient
	Volume of sample

Determination of SOD: The assay mixture consisting of 0.1 ml 312 µM NBT and 10µl 120 µM PMS were incubated for 10 minutes at 25°C. Then, 10µl RBC lysate was added and the reaction was initiated by addition of 0.1 ml 936 µM NADH. The absorbance was recorded at 560 nm for 2 minutes at 60 seconds interval. A control was also

run simultaneously. The SOD activity was calculated by the following formula:

$$\text{Calculations: SOD (U/g Hb)} = \Delta C / 2 \times \text{Hb in RBC lysate}$$

Where,

ΔT - Change in OD of Test at 60 seconds interval.

ΔC - Change in OD of Control at 60 seconds interval.

Feed analysis: Feed samples in each group were analyzed for various ingredients as mentioned in Table 1. The composition of ingredients was estimated by a Bureau of Indian Standards approved government laboratory (Punjab Biotechnology Incubator, Mohali, Punjab). The analyses included total fatty acids (method 972.28), fatty acid profile (method 963.22), free fatty acids (method 940.28), moisture (method ca 2a-45), insoluble impurities (method ca 3-46), unsaponifiable matter (method 933.08) and peroxide value (method 965.33) under the active oxygen method (method cd 12-57).

Statistical analysis

Data were analyzed according to a randomized complete block design by using MIXED model equation methods with SAS (statistical analysis system, version 9.3, USA) program. The effects of treatments on antioxidant status in sows and live litter weight and weight gain in piglets were evaluated by repeated measures analysis of variance. Due to lack of normality, the data were transformed to algorithmic scale. Tukey-Kramer adjustment was used for multiple comparisons of differences among all dietary treatments, including the control diet. All data presented were expressed as least squares means with their standard errors (SE). For all analyses, a confidence level of $P < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Impact of crushed flaxseed supplementation on LPO:

Changes in LPO were assessed by the amount of end product malondialdehyde (MDA) produced as shown in Fig. 1. The MDA levels were high on the first day of sampling (day-15), followed by a drop ($P < 0.05$) at current farrowing (day 0) and remained fairly constant during the lactation period (day 7-28) in all the sows. During the gestation period (day 30-90), a rise in levels of MDA were observed in all the groups. MDA activity was significantly ($P < 0.05$) affected by the day of gestation with MDA higher at day 30 of gestation with further rise ($P > 0.05$) at day 60 and highest ($P < 0.05$) at day 90. These observations are in

consonance with the findings of earlier studies (Herrera and Ortega-Senovilla, 2010, Kim *et al.*, 2013) that during the gestation period, rapid growth and development of fetus leads to increased metabolic burden on sow, causing elevated systemic MDA and subsequently LPO. This MDA-induced oxidative stress exacerbates maternal systemic inflammatory response to pregnancy and aberrant cytokine expression and vice versa (Burton and Jauniaux, 2011). Nevertheless, high levels of MDA during gestation period might imply a higher however, balanced antioxidative capacity of pregnant sows and thus a better protection against oxidative damage especially during critical period in the present study (Toescu *et al.*, 2002). Supplementation of flaxseed to sows failed to impact MDA concentrations at any of the days measured both during the lactation period as well as during the gestation period ($P>0.05$; Fig. 1).

Similarly, including 1.0% Echium oil (source of stearidonic acid) and 1.0% linseed oil in the sow diet from day 73 of gestation and during lactation did not affect MDA level in sows at day 93 of gestation and at farrowing (Tanghe *et al.*, 2013). Likewise, feeding linseed oil (0.5%) or fish oil (0.5%) to sows from day 45 of gestation until weaning did not reveal any signs of altered MDA in sows during pregnancy (day 93; Tanghe *et al.*, 2015). In another study, no effect on MDA was observed in sows during the gestation period (day 84) and at farrowing subsequent to dietary supplementation with fish oil from mating until the day of farrowing (Luo *et al.*, 2019). On the contrary,

sows fed high concentrations of linseed oil (2%) or fish oil (2%) from day 45 of gestation until weaning resulted in higher MDA levels at farrowing (Tanghe *et al.*, 2015). Similar observations (increased MDA concentrations in plasma, colostrum and milk of sows) were also recorded by Shen *et al.* (2015) who fed even higher content of fish oil (4.8%) in sow diet in late gestation (day 84) and lactation (day 21). Furthermore, feeding high levels of fish meal (3.0% or 5.0%) starting at day 85 of gestation and continuing through lactation also increased ($P<0.05$) the plasma MDA levels in sows during gestation and lactation (Yang *et al.*, 2019). The suggested reason was that feeding flaxseed or fish oil (linolenic acid) at higher ($>2.0\%$) levels is believed to offset homeostasis, incite a stress response, decreases resistance to stress and make the animal susceptible to oxidation of fatty acids that can lead to suppression of the immune system (Grundt *et al.*, 2003, Tanghe *et al.*, 2015). Nonetheless, no effect of flaxseed supplementation on LPO could be ascertained in the current study which might be due to prolonged supplementation of flaxseed to sows that may lead to adaptive response to oxidative stress as seen in the present study. Long-term feeding of PUFA failed to affect LPO in sows (Fernández-Dueñas, 2009) and human beings (Hasena *et al.*, 1998).

Impact of crushed flaxseed supplementation on superoxide dismutase: The superoxide dismutase (SOD) activity in sows is given in Fig. 2. Fifteen days prior to start of the treatment, the SOD activity exhibited no difference

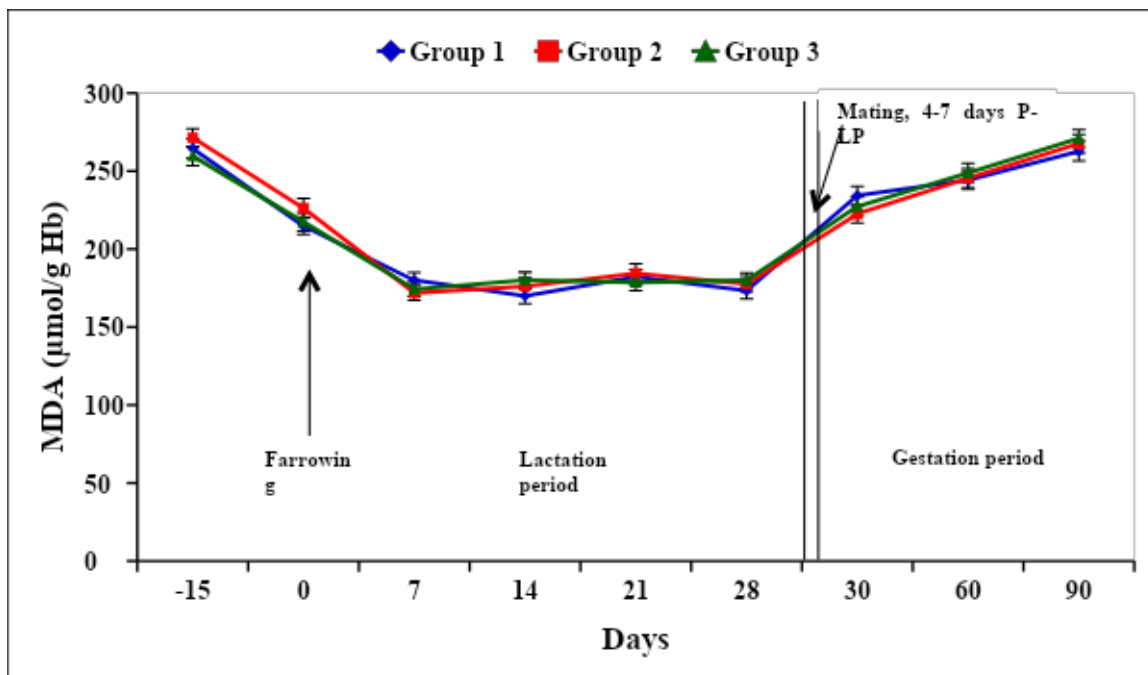


Fig. 1: Plasma malondialdehyde (MDA) concentrations ($\mu\text{mol/g Hb}$; Mean \pm SE) during lactation and gestation period in sows. Group 1 (Control, $n=7$), Group 2 (0.5% FS, $n=7$) and Group 3 (1.0% FS, $n=7$). Abbreviation: P-LP: Post-lactation period, FS: Flaxseed supplementation.

between the groups. A short rise ($P<0.05$) in the activity of SOD was observed on the day of farrowing (day 0). No changes in the SOD activity were observed from farrowing (day 0) through the lactation period (day 28) in sows of all the groups. A considerably lower ($P<0.05$) activity of SOD was noticed during gestation period (day 30-90) in sows. During pregnancy, females are highly susceptible to oxidative stress and SOD is the first enzyme involved against free radicals to reduce oxidative stress (Yu, 1994; Herrera and Ortega-Senovilla, 2010). Elevated systemic oxidative stress during the late gestation period reduced antioxidant enzyme activity in highly prolific sows (Berchieri-Ronchi *et al.*, 2011). In the current study, loss of intracellular activity of SOD indicated that it could be utilized as a result of enhanced defense mechanism to reduce the load of oxygen radicals in pregnant sows. Supplementation of flaxseed to sows did not influence the SOD activity as revealed by absence of difference in the SOD levels during lactation and gestation in the three groups in the present study (Fig. 2). Likewise, supplementation with fish oil (4.8%) to sows failed to affect plasma SOD levels in late gestation (day 84), farrowing (day 0) and lactation (day 10 and day 21; Shen *et al.*, 2015). Similarly, Luo *et al.* (2019) observed no impact on SOD activity during late gestation (day 84) subsequent to dietary supplementation of sows with fish oil (2.5%) from mating until the day of farrowing. Nevertheless, failure of flaxseed supplementation to affect the SOD activity in sows could be due to long-term feeding of flaxseed in the

current experiment. Previous reports in sow (Fernández-Dueñas, 2009) and in human beings (Hasena *et al.*, 1998) have also suggested that continuous intake of n-3 PUFA for longer duration has little or no effect on enzymatic antioxidant system in plasma.

Impact of crushed flaxseed supplementation on current litter performance: The average total live weight and weight gain in litters of all sows during the lactation period are summarized in Figs. 3-4. The average live litter weight was similar at birth (after cross-fostering) in all the groups. From day 0 until day 28 of lactation there was a steady increase ($P<0.05$) in live litter weight as well as weight gain in all the groups (Figs. 3-4). These findings are in consonance with the observations of previous studies (Mateo *et al.*, 2009; Lavery *et al.*, 2019) that body weight of nursing piglets increases with increasing growth as their maintenance energy requirement and milk intake increases. No effect of dietary flaxseed supplementation to sows on their litter weight and weight gain were noticed until day 21 of lactation. A treatment effect on litter weight as well as weight gain was observed on day 28 of lactation when higher ($P<0.05$) values of both variables were recorded in group 2 and group 3 than in group 1 (Figs. 3-4). Reports regarding the effects of flaxseed supplementation (n-3 PUFA) in sow diet on piglet weight and growth are inconsistent. While some studies (Mateo *et al.*, 2009; Smit *et al.*, 2013; Pedersen *et al.*, 2016) have shown that

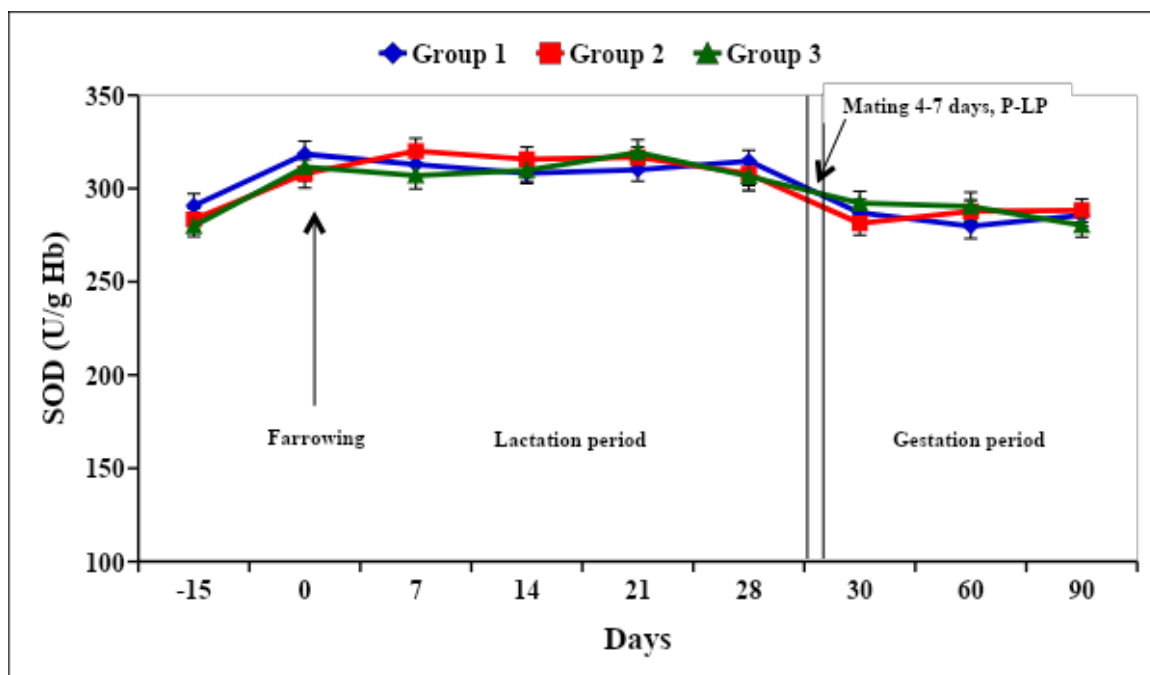


Fig. 2: Plasma superoxide dismutase (SOD) concentrations (U/g Hb; Mean \pm SE) during lactation and gestation period in sows. Group 1 (Control, n=7), Group 2 (0.5% FS, n=7) and Group 3 (1.0% FS, n=7). Abbreviation: P-LP: Post-lactation period, FS: Flaxseed supplementation.

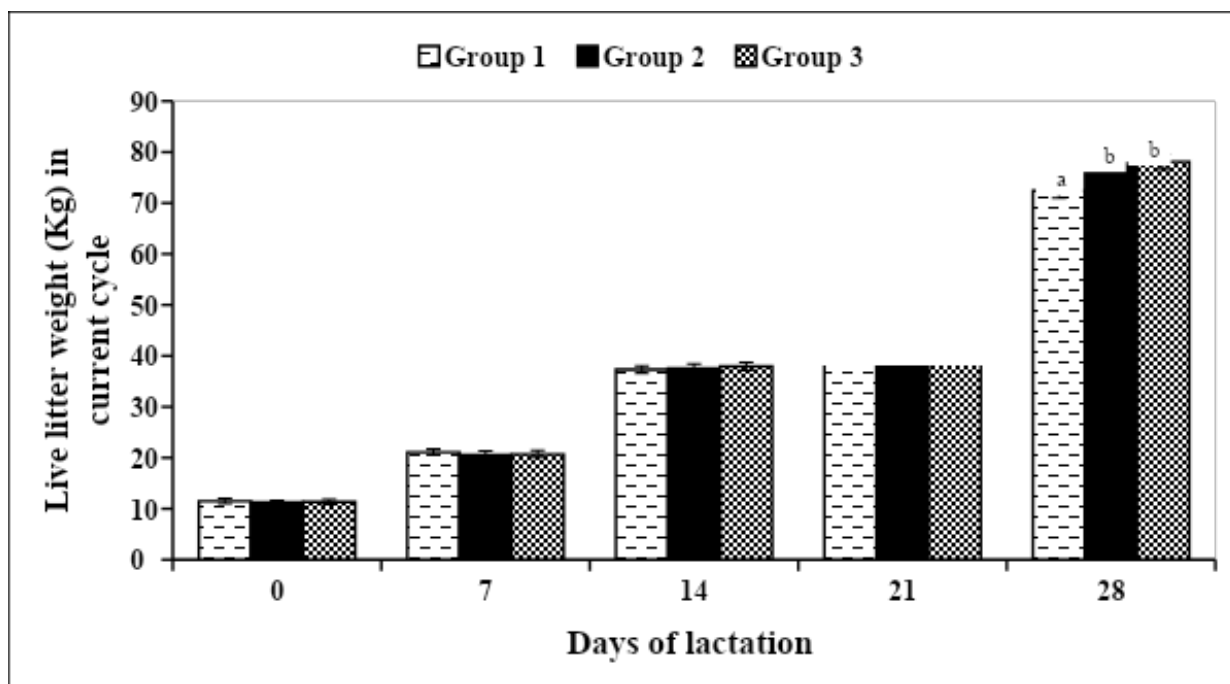


Fig. 3: Live litter weight (Kg; Mean \pm SE) during lactation period in current production cycle of sows. Group 1 (Control, n=7), Group 2 (0.5% FS, n=7) and Group 3 (1.0% FS, n=7). Abbreviation: P-LP: Post-lactation period, FS: Flaxseed supplementation. Values with different alphabetic superscripts differ significantly ($P<0.05$) from corresponding values in the three groups.

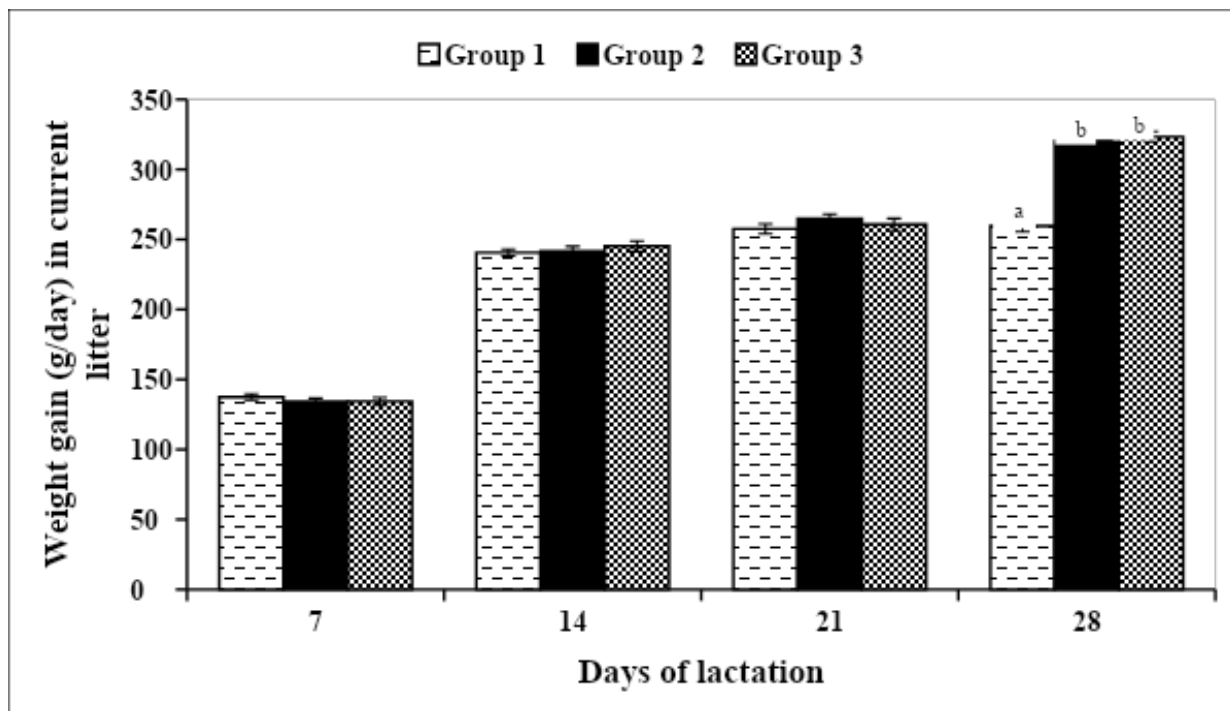


Fig. 4: Feed intake (Kg/day; Mean \pm SE) during lactation period in current production cycle of sows. Group 1 (Control, n=7), Group 2 (0.5% FS, n=7) and Group 3 (1.0% FS, n=7). Abbreviation: P-LP: Post-lactation period, FS: Flaxseed supplementation. Values with different alphabetic superscripts differ significantly ($P<0.05$) from corresponding values in the three groups.

supplementation of n-3 PUFA to sow diet in late gestation and throughout lactation increased litter weight toward late lactation, others (Kruse *et al.*, 2011; Lavery *et al.*, 2019) reported no impact of n-3 PUFA supplementation to sows from day 107 of gestation to weaning on piglet or litter weight at birth and weaning. The suggested reason in these studies regardless of dietary treatment was higher (7.1 kg/day) feed intake in sow during lactation than that found in commercial herds. In another study, flaxseed supplemented in milk diet had a positive correlation with the body weight of weanling gilts (Vlčková *et al.*, 2018). Similar impact on body weight (an increase of 70-80%) was observed in a previous study on gnotobiotic piglets supplemented with probiotics and flaxseed oil (Nemcová *et al.*, 2012). Nevertheless, positive effect on litter weight post-weaning, if any, in the current study was not recorded since this study terminated at weaning for current litters in all the sows.

CONCLUSIONS

It can be concluded that dietary flaxseed had no effect on malondialdehyde and superoxide dismutase concentrations in sows and body compositional variables in piglets.

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CONFLICT OF INTEREST

The authors declare no conflict of interest in the conduct of this experiment.

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